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THE QUEENSLAND JOURNAL OF AGRICULTURAL SCIENCE

VOL. 8. NO. 1.

MARCH, 1951.

A WILT DISEASE OF THE PASSION VINE (*PASSIFLORA EDULIS*) CAUSED BY A SPECIES OF *FUSARIUM*.

By T. McKNIGHT, M.Sc., Pathologist, Science Branch, Division of Plant Industry.

SUMMARY.

The symptoms of natural and artificial infection of passion vine with a species of Fusarium causing a wilt disease of the host are described.

In inoculated seedlings a vein clearing symptom in the youngest unfolded leaf is shown and leaf shedding occurs.

Tests with small numbers of plants showed Passiflora alba and P. suberosa to be susceptible to the fungus. Some resistance was shown by P. foetida, P. flavicarpa, and a proportion of plants of one strain of P. edulis.

It is suggested that a practical solution to the problem may be secured either by selecting resistant individuals of P. edulis or by top-grafting P. edulis to a root system of a suitable resistant species of Passiflora.

The species of Fusarium causing wilt disease is distinct from F. bulbigenum.

INTRODUCTION.

Fusarium wilt disease of the passion vine (*Passiflora edulis* Sims.) produces severe economic losses in plantations in south-eastern Queensland, where the greater portion of Queensland's passion vine industry is located. In one district, Mount Cotton, which was previously a prosperous passion vine area, the culture of the crop has ceased as the result of recurring epidemics of wilt disease.

The disease has so far been confined to a wilting of more or less advanced plants in the field and has not been detected in growers' seed-beds. It has, however, been demonstrated experimentally that infection with an unnamed species of *Fusarium* in the seedling stage leads to death. It is probable that infection in the field originates at or near the site of the vine, for it is unlikely that plants infected in the seed-bed would remain symptomless until they were reaching maturity.

SYMPTOMS.

The end point of the disease is the complete collapse of the plant (Figure 1). Plants may die in positions at random through the plantation, or several may die in adjacent positions within the row, suggesting spread of the fungus by root contact. The wilting is sudden and generally involves the whole of the plant. Occasionally a collapse of one side of the vine precedes the wilting of the whole vine. A previously symptomless plant may collapse in 24-48 hours.

In wilted plants there is a prominent browning in the vascular system of the root, crown, and leaders. The vascular discolouration in the leaders may extend as far as seven feet from ground level.

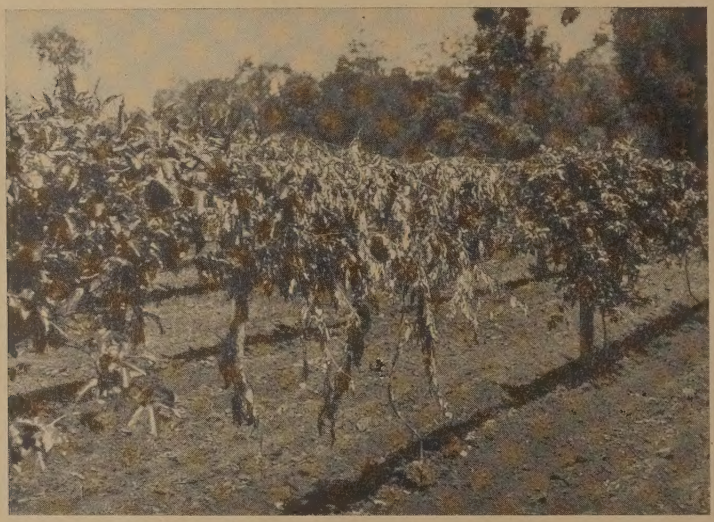

Figure 1.

Collapse of Passion Vine in the Field due to Infection with *Fusarium* sp.

While the symptoms of the disease in the field are more or less typical of those shown in other plants infected with species of *Fusarium,* the following symptoms of infected seedlings are worthy of note.

The youngest unfolded leaf of species of *Passiflora* inoculated with the causal *Fusarium* shows a clearing of the veins which produces "islands" of green interveinal areas. This is an early symptom of Fusarium wilt preceding leaf shedding and is commonly detected within seven to nine days after inoculation.

A general wilt of the seedlings, with all leaves intact, occurred only rarely in tests with seedlings. Leaf abscission is the main symptom when

seedlings are inoculated with the wilt fungus in the greenhouse. This occurs within a fortnight after inoculation, and commences with the loss of the cotyledonary leaves.

The disease commonly appears in plantations on old cultivations following various fruit and truck crops, but has also been recorded on new land. The wilt has been observed in February, May, June, July, September, October and November. The disease manifests itself before the vines are one year old; records of its occurrence have been made in vines varying in age from four to 10 months.

PATHOGENICITY TRIALS.

A *Fusarium* was isolated from the wilted passion vine shown in Figure 1 and used in inoculation experiments as isolate No. 6905B.

Inoculations were made with 4-6 weeks' old subcultures of the isolate on potato-dextrose-agar; seedlings were inoculated by either the soil-slurry method, in which the spores and mycelium were scraped from the surface of the medium and added to a mixture of soil and water, or by the water-fungus method, in which the inoculum was added to sterile water. The seedlings used for the experiments were grown in a greenhouse and generally had 1-3 true leaves. They were removed from the pots, their roots washed in tap water, dipped and agitated in the inoculum, and the seedlings then replanted. Control plants received the same treatment without the addition of the fungus.

Figure 2.

Inoculation of Seedlings.—Right, not inoculated; left, seedlings collapsing 15 days after inoculation.

Using the soil slurry-fungus method, of 43 seedlings of the ordinary variety of *P. edulis* inoculated, 41 wilted, and of 16 inoculated by the water-fungus method, 16 wilted.

Table 1 summarizes the results obtained from greenhouse pot experiments.

Table 1.

INOCULATION OF SPECIES OF *Passiflora* WITH *Fusarium* ISOLATE 6905B.

Date of Inoculation.	Species.	Inoculated.		Not Inoculated.		Period in days for symptoms to appear.
		Negative.	Positive.	Negative.	Positive.	
4–11–47 ..	*P. edulis*	0	18	18	0	10–13
17–11–47 ..	*P. edulis*	2	23	27	0	13–22
26–11–47 ..	*P. edulis*	0	16	16	0	13
10–2–48 ..	*P. alba*	0	12	11	0	9–16
	P. suberosa	1	9	10	0	9–14
	P. foetida	11	0	10	0	..
	*P. flavicarpa** ..	4	0	3	0	..
3–3–48 ..	*P. edulis* (Hackett's Mammoth)	3	6	10	0	12–27

* In a subsequent trial carried out by G. Purss with *P. flavicarpa*, 29 out of 50 seedlings wilted within 29 days (Departmental records).

In areas where Fusarium wilt of the passion vine occurs, the most noteworthy Fusarium wilt diseases of other crops are that of tomato caused by *F. bulbigenum* Cooke and Massee and that of banana caused by *F. cubense* E.F.S. To determine whether any relationship existed between the passion vine and tomato wilt organisms, the cross inoculations listed in Table 2 were carried out, using the water-fungus method and 16 plants per treatment. After four weeks the condition of the plants in each treatment was as stated in the table.

Table 2.

COMPARISON OF THE PATHOGENICITY OF THE PASSION VINE ISOLATE WITH *F. bulbigenum*.

Organism Used.	Passion Vine.	Tomato.
Fusarium isolate 6905B	16 dead ..	16 healthy
F. bulbigenum (C.S.I.R.O. normal tomato testing strain)	16 healthy	16 dead or stunted
Uninoculated	16 healthy	16 healthy

DISCUSSION.

The small-scale inoculation tests have shown that there may be some resistant individual plants amongst strains of *Passiflora edulis* (e.g., "Hackett's Mammoth") and also that *P. foetida* and *P. flavicarpa* show some resistance to the fungus.

Such differential resistance suggests the commercial possibility of grafting *P. edulis* scions onto resistant stocks if interspecific compatability can be established.

NEW GENERA AND SPECIES OF DACINAE (TRYPETIDAE, DIPTERA) FROM QUEENSLAND.

By A. W. S. MAY, M.Agr.Sc., Entomologist, Science Branch, Division of Plant Industry.

SUMMARY.

Two new genera—Neozeugodacus (genotype, N. aureus) and Diplodacus (genotype, Dacus signatifer Tryon)—have been established.

New species described in addition to Neozeugodacus aureus are Strumeta mutabilis and Afrodacus mesoniger.

INTRODUCTION.

During studies concerned with the ecology, recording of native hosts and control of the economic species of fruit flies occurring in Queensland, three new species of Dacinae have been discovered. Two of these have been bred from their native hosts, while all three were caught in lure traps placed in either citrus or deciduous fruit orchards. This latter phenomenon does not necessarily suggest their association with cultivated fruits, for other species of no economic importance are consistently taken in traps over the period when their native hosts are abundant. To facilitate the compilation of a comprehensive host list of the Dacinae occurring in this State, these new species are described.

A revision of the host lists recorded for those species of fruit flies already recognized in Queensland provided an opportunity to examine Tryon's species *Dacus signatifer*. An examination of material bred from the type locality revealed that the characters of this species do not agree with those of any known genus. A new genus for this species is therefore described.

STRUMETA Walk.

Although a series of 40 specimens was available when describing the undermentioned species, its correct generic status is a matter for conjecture. Considerable variation was found in the development of the *a.sa.* bristle. A small percentage of the series had no bristle and with other generic characters could be placed in the genus *Afrodacus* Bez. In those specimens, which would go into the genus *Strumeta* on the basis of the *a.sa.* bristle, this feature showed considerable variation, ranging from weak and almost imperceptible, under high magnification, to strong.

This variation within a species immediately brings into question the correct status of many of the species now recognized, particularly when it is known that some have been erected and classified generically from one specimen, or at the most only a few. The validity of the generic classification for the Dacinae is similarly in doubt, but a full appreciation of this controversial subject can only be arrived at after a detailed statistical study of large numbers of each species. Admittedly, most of the species conform more closely to the pattern of

characters associated with their genus, but the confusing variation associated with the species described below calls for a review of their status. For the present, however, the existing system of classification will be followed.

The species under consideration has been placed in the genus *Strumeta* because the other essential characters are present. These include 2 *sc.* and 2 *prsc.* bristles, the third abdominal tergite of male ciliated, a supernumerary lobe in the wing of the male, and the basal segment of the ovipositor shorter than the abdomen.

Strumeta mutabilis n.sp.

Female:—Length, excluding ovipositor, 6·2 mm.; wing 5·8 mm.

Male:—Length, 6·0 mm.; wing 5·5 mm.

Head:—*Frons* fulvous, length one and a-half width; pale yellow above lunule and anteriorly along sides; orbital spots and roughly triangular antero-medial blotch, dark brown; vertical calli shiny; bristles: 1 *s.or.*, 2 *i.or.* (may be three pair in some specimens). *Lunule* dark brown. *Antennae* fulvous except

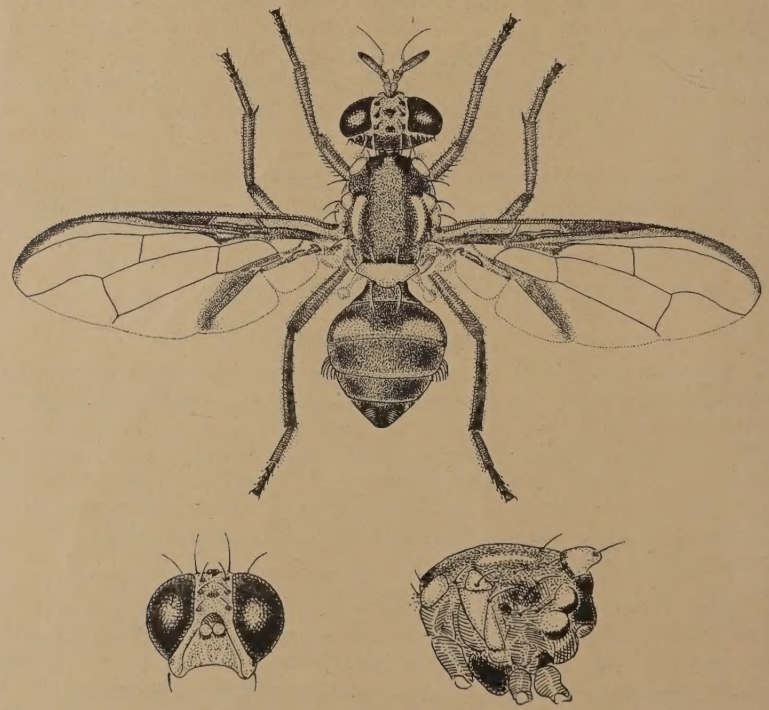

Figure 1.

Strumeta mutabilis.—Adult male (× 10); head of male (× 12); thorax of male (× 12).

[*Drawings by William Manley.*

outer surface and inner apical portion of 3rd, dark brown; covered with short white hairs; 3rd segment almost three times 2nd, latter with short brown dorsal bristle; arista long, brown except basal portion fulvous. *Face* pale fulvous, shiny, with small dark brown spot beneath base of each antenna, whitish between ptilinal suture and eye; no facial spots (28 specimens had no facial spot while the remaining 12 showed variation between a faint spot or blotch on one or both sides to a distinct but small spot on each side). *Palps* fulvous, rounded, flattened, may be somewhat brownish on margins. *Genae* pale with brown subocular spot; bristle well developed, black. *Occiput* fulvous, occipital row weak, approximately five on each side. *Vertex* fulvous, brown along transverse carina, ocellar triangle black; bristles: ocellar present, very weak; postverticals strong; all dark brown to black.

Thorax:—Uniformly red-brown, medially greyish dust, short hoary pubescence, except for following; black: a pair of spots on anterior margin of mesonotum which may be slightly attenuated backwards towards suture, indefinite blotches both above and below postsutural stripes and in front of scutellum, sternopleura, above hind coxae and on lower portion of hypopleurals; yellow: humeri, notopleurals, a subrectangular mesopleural stripe extending on to sternopleuron and with upper anterior margin commencing midway between notopleural and humeral calli, 3/4 of upper and 2/3 of lower hypopleurals, pair of narrow lateral post sutural stripes narrowing posteriorly and ending well before upper *p.sa.* bristle; with the following dark brown (or brown where stated) bristles: *scp.* 4 (central pair brown, weaker), *npl.* 2, *mpl.* 1 (brown), *a.sa.* 1, *p.sa.* 2, *prsc.* 1 pair, *pt.* 1 (weak, pale), *sc.* 1 pair (apical, brown). (Note.—The *a.sa.* bristle may vary both in length and thickness. Though entirely lacking in a few specimens, in the majority of specimens examined it was poorly developed, recumbent and difficult to discern. In some cases it was present on one side only). *Postnotum* black except for medial longitudinal yellow brown band widening anteriorly. *Scutellum* yellow with dark brown band on anterior margin. *Legs* pale fulvous except for hind tibiae dark brown and mid and hind coxae and extremities of tarsi, fulvous; apical spur on middle tibiae. *Wings*: Costal cell fulvous. Costal band fuscous, occupying stigma, most of R_1, extremity of R_{2+3} and extending to a point midway between extremities of r_{4+5} and m_{1+2}; remainder of cell R_1 and above median cell fulvous; anal streak fuscous; *r–m* cross vein oblique and slightly sinuate, the section of m_{1+2} before and after it being in the proportion of 1·75; cell Cu restricted basally, its length in proportion to cu_1 + 1a being 1·5 in female and 2·3 in male. Supernumerary lobe present in male.

Abdomen:—More or less uniformly yellowish brown (in some specimens 4th and 5th tergites blotched with dark brown); posterior half of 2nd tergite, except for medial portion, somewhat paler; posterior margins of 2nd, 3rd and 4th pale fulvous bordered anteriorly on 3rd and 4th by thin dark brown discontinuous line. Shining spots on 5th brown. No medial stripe. *Ovipositor* shiny, brown, long. *Pecten* on 3rd tergite of male.

Holotype ♂, allotype ♀, 5 ♂♂ and 33 ♀♀ paratypes ex lure traps in deciduous orchards at Stanthorpe, Warwick, Toowoomba and in a citrus orchard at Gatton, Queensland, September, October and November, 1949 and 1950. (A. W. S. May.) Types in Queensland Museum; holotype Reg. No. T.5265, allotype Reg. No. T.5266.

This species somewhat resembles *S. tryoni* (Frogg.) and *S. gurneyi* (Perk.) in general coloration and in having coloured costal cells. It can be differentiated from the former as follows:—

S. mutabilis.	*S. tryoni.*
Virtually no facial spots	Large facial spots
Abdomen of uniform colour, no medial stripe..	2nd tergite whitish on posterior half. Faint medial stripe
Costal band ending midway between r_{4+5} and m_{+2}	Costal band ending just beyond r_{4+5}
5th tergite with shining brown spots	Spots not conspicuous

S. gurneyi can be more readily distinguished by the brown markings on the scutellum, the presence of a medial stripe on the abdomen and the black shining spots on the 5th abdominal tergite.

AFRODACUS Bez.

The species described below has been provisionally placed in this genus, but until a male has been examined its correct status cannot be determined. Such characters as no *a.sa.* bristle, 2 *prsc.* and 2 *sc.* bristles, together with a flattened ovipositor, support this classification.

Afrodacus mesoniger n.sp.

Female:—Length, excluding ovipositor = 6·0 mm.

Head:—*Frons* fulvous, length twice width; orbital spots dark brown, brown medial blotch present in some specimens; bristles: 1 *s.or.* and 2 *i.or.* all black. *Lunule* dark brown. *Antennae* brown, darkening towards extremity of 3rd; 2nd with pale brown dorsal bristle; 3rd slightly more than three times length of 2nd; arista black except for basal third fulvous. *Face* fulvous, shiny, with subcircular black spot on inner margin of each antennal groove; pale fulvous along margin of eyes. *Genae* fulvous with faint brown subocular spot; genal bristle pale, weak. *Palps* flattened, fulvous. *Occiput* uniformly brown; postvertical bristles strong, black.

Thorax:—Mostly black*, punctulate with short hoary pubescence, except: pair thin longitudinal vittae from inner margins of humeri to scutellum, a thin

* Newly emerged specimens show considerable variation in the extent of black pattern on the mesonotum and the shade of colouration of the abdomen. Generally, the central portion of the mesonotum appears black, covered with a hoary pubescence, as in the type (field specimen), but newly emerged flies may have the central portion brown except for a median black vitta. Graduations between these two extremes could be expected. For a field specimen, the general colour of the tergites is orange-brown, but for newly emerged flies these areas are fulvous.

medial line, a vitta adjacent to inner margins of each postsutural stripe, all shiny black; brown: on anterior margin both above and below humeral calli, between humeral and notopleural calli, immediately below and behind post sutural stripes; yellow: humeri, notopleurals, subrectangular mesopleural stripe extending slightly on to sterna, upper two-thirds of both hypopleural calli, the

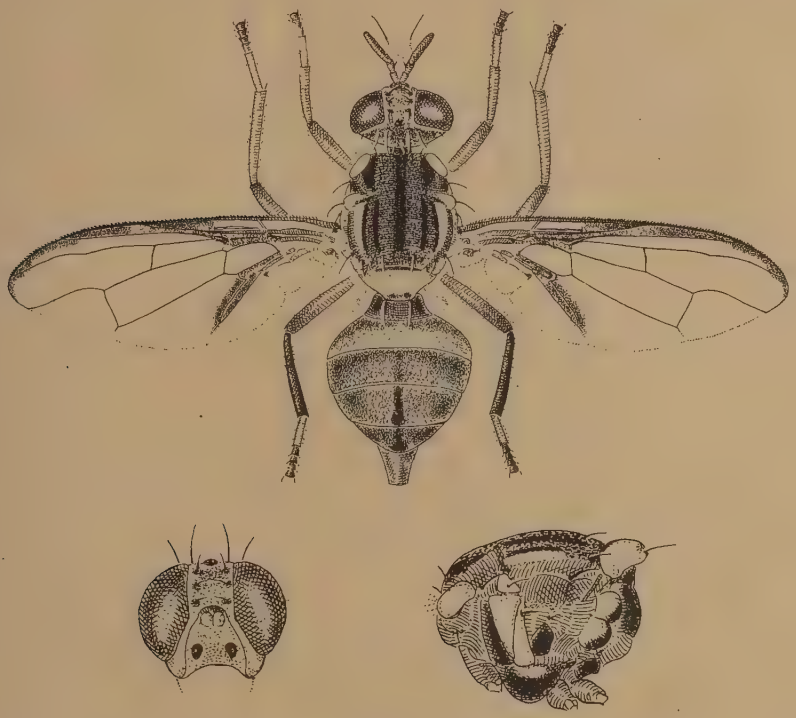

Figure 2.

Afrodacus mesoniger.—Adult female (× 9); head of female (× 12); thorax of female (× 12).

[*Drawings by William Manley.*

lateral post sutural stripes, which taper posteriorly and end well in front of upper *p.sa.*; bristles: inner and outer *scp.*, *npl.* 2, *mpl.* 1 (reclinate, thin), *pt.* 1 (weak), *p.sa.* 2, *prsc.* 1 pair, *sc.* 2, all dark brown or black. *Scutellum* yellow with black anterior border and dark brown spot between bristles. *Postnotum* black, hoary. *Legs* fulvous except hind tibiae dark brown, and middle and hind coxae brown. *Wing*: length 5·8 mm., hyaline except for narrow fuscous costal band occupying stigma, cell R_1, outer margin of cell R_{2+3} and finishing midway between extremities of r_{4+5} and m_{1+2}. That portion immediately above median cell fuscous. Anal streak fuscous. *r–m* cross vein oblique. Costal cells colourless.

Abdomen:—Generally broad. Tergites: 1st dark brown. 2nd with anterior half orange-brown, posterior half pale fulvous narrowing laterally, covered with whitish hairs. 3rd-5th orange brown, blotched dark brown to black anterior margin 3rd and dark brown blotches lateral margins 5th: no shining spots on 5th; distinct broad medial black stripe on 4th and 5th which may extend indefinitely on to 3rd. *Ovipositor*: fulvous, flattened.

Holotype ♀, 1 ♀ paratype ex. lure traps in mixed deciduous and citrus fruit orchard, Toowoomba, Queensland, April, 1950 (A. W. S. May).

4 ♀ ♀ paratypes from fruits of *Neolitsea involucrata* (Lam.) Alston, Cunningham's Gap, Queensland, February, 1951. (A. W. S. May.)

Type in Queensland Museum, Reg. No. T.5264.

This species resembles *A. javanensis* Perk. in having a black thorax but differs in the markings on the face, the wing pattern, the relative absence of black markings on the legs and the colour pattern on the abdomen. It can be distinguished from *A. jarvisi* (Tryon) and *A. brunneus* Perk. and May by the black thorax, yellow markings of the mesonotum and the absence of infuscation on the *r–m* cross vein.

NEOZEUGODACUS n.g.

Genotype:—*Neozeugodacus aureus* n.sp.

A Dacinae bred from the fruits of *Alangium villosum* (Bl.) Wangerin ssp. *tomentosum* (F. Muell.) Bloembergen var. *australe* Bloembergen does not conform to any of the known genera and the above new genus has been created to enable its description. It is close to *Parazeugodacus* Shir. but differs in the absence of the anterior supra-alar bristle.

The characters of the genus can be defined as follows:—Dacinae with four scutellar, no anterior supra-alar, two post supra-alar, no humeral and two pre-scutellar bristles; a row of post lateral cilia on the third abdominal tergite of male; no supernumerary lobe in the wing of the male; antennae not longer than the face, basal segment of ovipositor shorter than abdomen.

Neozeugodacus aureus n.sp.

Female:—Length, excluding ovipositor, 6·8 mm.; wing 6·0 mm.

Male:—Length 6·6 mm.; wing 6·1 mm.

Head:—*Frons* pale fulvous fading to whitish along anterior border and above lunule; length one and a half times width; brown triangular spot at base of each *s.or.* bristle; anterior medial brown patch somewhat darker towards centre, may be triangular; 1 *s.or.*, 3 *i.or.* bristles. *Face* glassy, pale fulvous, whitish pubescence between ptilinal suture and eye; facial spots black, semi-circular. *Lunule* pale, shiny. *Genae* whitish to very pale fulvous with distinct brown subocular spot; genal bristle black, well developed. *Antennae* fulvous, covered with short whitish hairs; 2nd segment with brown dorsal bristle; 3rd

segment brown on outer surface, almost three times 2nd; arista dark brown, basally fulvous. *Palps* pale fulvous, flattened, rounded apically, with basal brown blotch. *Occiput* fulvous, centrally blotched brown, occular margin paler, almost whitish; occipital row weak, variable, 4-6 each side. *Vertex*: ocellar triangle brown; post verticals strong, black; weak ocellar bristles.

Figure 3.

Neozeugodacus aureus.—Adult male (× 10); head of male (× 12); thorax of female (× 12).

[*Drawings by William Manley.*

Thorax:—Fulvous. On the mesonotum a central greyish area extending from anterior margin to a point approximately midway between suture and scutellum, divided medially and bounded on each side by shiny brown vittae; the medial vitta widening anteriorly and posteriorly. The entire mesonotum covered with a golden appressed pubescence somewhat denser in front of suture; a thin dark brown line along suture and in front of mesopleural stripe; white hoariness on sternopleura. With the following yellow markings: humeral and notopleural calli, latter small; a triangular mesopleural stripe with base extending from humeral to notopleural callus with apex truncate and ending on upper sternopleuron, anterior margin concave; 5/6 upper and 3/4 lower

hypopleura (remainder fulvous); a pair of parallel sided post sutural stripes ending just before upper *p.sa.* bristles. *Scutellum* yellow except for posterior half and anterior margin dark brown. *Postnotum* fulvous except for thin brown line along upper margin. *Halteres* pale yellow. *Bristles*: *scp.* 4 (inner pair weak), *npl.* 2, *mpl.* 1 (reclinate), *pt.* 1 (weak, pale), *p.sa.* 2 (upper pair weaker than lower), *prsc.* 1 pair, *sc.* 2 pair, the apical pair strong and parallel, the basal pair weak. All dark brown to black. There is no *a.sa.* bristle. *Legs* pale fulvous except hind tibiae brown and tarsi darker tending brownish towards extremities; middle tibiae with shiny black apical spur; coxae fulvous. *Wings* hyaline. Costal cells fulvous; costal band fulvous to extremity of stigma, remainder fuscous, occupying cell R_1, the outer margin of cell R_{2-3} and ending just beyond r_{4-5} (in some specimens almost midway between r_{4+5} and m_{1-2}); fulvous above basal cell. Anal streak fuscous, broad. *r–m* cross vein slightly oblique, the section of m_{1-2} before and after it being in the proportion of 1·5. Cell Cu greatly restricted basally, its length in proportion to Cu_1 + 1a being 1·6 in the female and 2·6 in the male. A pale fulvous indefinite transverse band commencing at the upper end of the *r–m* cross vein, broadening to occupy the outer extremity of discal cell and narrowing sharply to end at the extremity of the Cu vein, the colour also fading towards the wing margin. No supernumerary lobe in the wing of the male.

Abdomen:—1st tergite deep fulvous; 2nd tergite fulvous on anterior third darkening laterally and extending down lateral margins, remainder whitish; remaining tergites pale fulvous to whitish medially but brown on lateral half of 3rd and 4th and extreme edge of 5th. A broad brown medial stripe commencing on anterior margin of 2nd and extending to posterior margin of 5th, becoming narrower and darker on 4th and 5th. Shining spots fulvous, not conspicuous. Ovipositor broad, flattened, shining, fulvous. Pecten on 3rd tergite of male. All tergites covered with appressed golden pubescence.

Holotype ♂, allotype ♀, 15 ♂ ♂ and 16 ♀ ♀ paratypes, bred from fruits of *Alangium villosum* ssp. *tomentosum* var. *australe*. Ravensbourne, Queensland, February, 1950 (A. W. S. May). Types in Queensland Museum: holotype Reg. No. T.5262, allotype Reg. No. T.5263.

DIPLODACUS n.g.

Genotype:—*Dacus signatifer* Tryon.

Tryon, in his description (1927) of this species, cites generic characters unrelated to *Dacus*. The presence of two pairs of scutellar bristles, a supernumerary lobe in the wing of the male and the absence of the anterior supra-alar bristle suggested that this species belonged to another genus.

This association of characters was confirmed when a series of specimens of this species was bred from the fruits of *Capparis lucida* R.Br.* collected at Bowen, March, 1950. This fruit fly is very similar in general colouration and markings to *Austrodacus cucumis* French, being also close generically in having

* Tryon wrongly recorded the host of this species as *Capparis laurifolia* R.Br.

no *a.sa.* or *prsc.* bristles. However, the presence of a pecten on the abdomen and a supernumerary lobe in the wing of the male separate it from *Austrodacus* Perk. It is closer to *Paradacus* Perk. and *Daculus* Speis. but differs from the former in having no *a.sa.* bristle and from the latter in having four scutellar bristles.

The characters of the genus are:—Dacinae with four scutellar, no anterior supra-alar, two posterior supra-alar and no prescutellar bristles; a pecten on the third abdominal tergite and a supernumerary lobe in the wing of the male; antennae not longer than the face, abdominal tergites not fused and the basal segment of the ovipositor shorter than the abdomen.

REFERENCE.

TRYON, H. 1927. Queensland fruit flies (*Trypetidae*), Series 1. Proc. Roy. Soc. Qld. 38: 176-223.

A LABORATORY WHIRLER FOR DETERMINING COUNTS OF THERMODURIC ORGANISMS IN RAW MILK SUPPLIES.

By V. R. SMYTHE, B Sc.Agr., Bacteriologist, Dairy Research Branch, Division of Dairying.

SUMMARY.

A laboratory whirler which permits the rapid performance of thermoduric counts on milk samples is described. The method is faster and more convenient than the orthodox roll-tube count and yet retains all the advantages of roll-tube counting.

INTRODUCTION.

The periodic occurrence of high counts of thermoduric organisms in some Queensland milk supplies has presented a problem in milk quality control work in this State. While health standards dictate that pasteurized milk shall contain not more than 50,000 organisms per millilitre, due attention must be paid to the numbers of organisms surviving pasteurization. When these numbers become excessive, improvement can be obtained by counting the thermoduric organisms in each farmer's supply and by conducting the necessary follow-up work on farms.

The large number of milk samples to be dealt with in work of this nature has caused some difficulty in the laboratory. The plate count was considered to be out of the question, since it is laborious, requires a large amount of equipment and overtaxes incubator space when large numbers of plates are involved. A solution to the problem has been obtained by the construction of a laboratory whirler which enables 300-400 counts to be done in tubes in a working day.

DESCRIPTION OF APPARATUS.

The whirling apparatus is featured in Figure 1. It has three vertical spindles, all running in ball bearings and covered with rubber to provide a friction drive of rubber on to rubber. The central spindle is directly coupled to the vertically-mounted motor. The two lateral spindles run in frames which can be adjusted into or out of drive with the central spindle by turning a knurled thumb screw. At the bottom of each lateral spindle is attached a rubber stopper which spins the tube by seating into its top.

Through each lateral frame slides a vertical stem situated behind the spindle. This stem is free to move vertically but is prevented from rotating by a ball-in-slot arrangement. It supports a foot plate bored with a half-inch hole to act as a simple bearing in which the bottom of the tube spins under water.

This stem is spring loaded above to keep the tube constantly and gently pressed upwards against the driving rubber stopper. Sufficient vertical movement is provided to enable the foot plate to be easily and quickly lowered for the replacement of tubes.

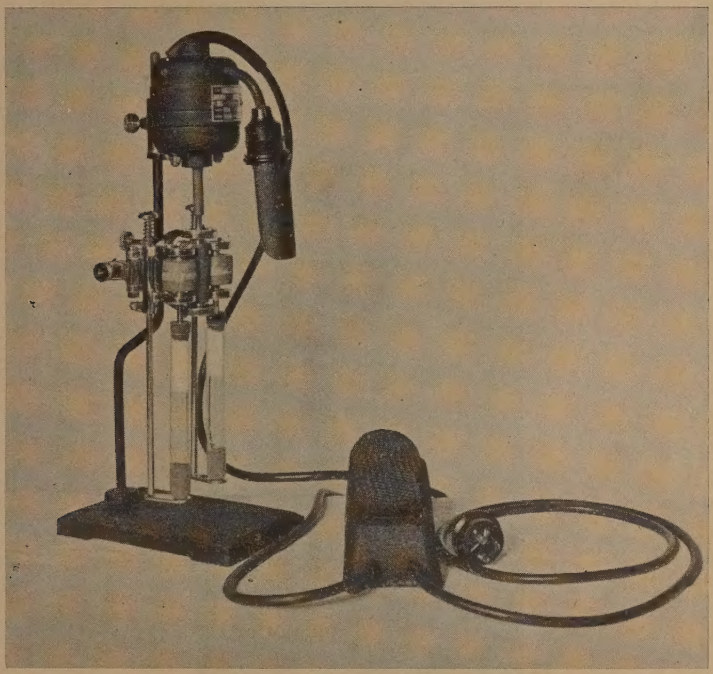

Figure 1.
Photograph of Whirler with Tubes in Position.

The whole apparatus is mounted on a sturdy stand on which it can be raised or lowered to the required height and to allow the vessel of water in which the tubes spin to be placed in position.

A small sewing machine motor operating on 240 v. A.C./D.C. and using 80 watts has provided ample driving power. This motor, when purchased, was equipped with a foot-type speed regulator which has been used with advantage to give the optimum speed of whirling.

METHOD OF PERFORMING COUNTS.

The media—tryptone, dextrose, yeast extract milk agar of the American Public Health Association (1948)—is dispensed in 4-5 ml. quantities in 6 in. $\times$ $\frac{3}{4}$ in. tubes plugged with an abbreviated cotton wool plug. Following autoclaving, the tubes should be covered to guard against contamination through the smaller cotton wool plug during storage.

After the media is melted and cooled to 45 deg. C., the tubes are inoculated with the sample of milk previously laboratory pasteurized. Inoculation is by a standard loop, calibrated gravimetrically to deliver ·01 ml. The neck of the tube and the cotton wool plug are flamed and the plug pushed down almost half an inch into the tube. The tubes are then placed in the whirler and whirled for 10-15 seconds in cold water. The high-speed whirling action thoroughly mixes the inoculum with the medium and throws the medium up on to the walls of the tube in an even layer before it quickly sets. Two spun tubes of media are shown in Figure 2. Tubes are incubated for 48 hours at the temperature employed for plate counting for pasteurized milk.

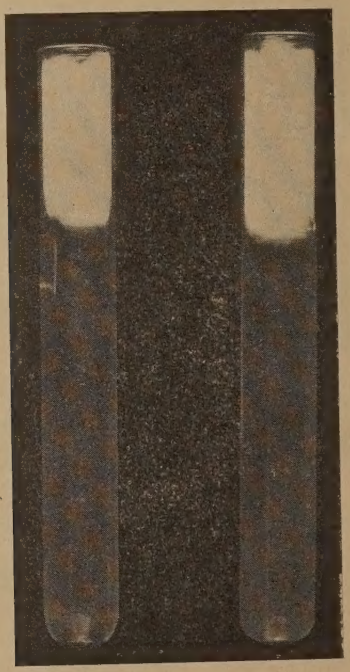

Figure 2.
Tubes After Whirling.

It is not necessary to count all tubes following incubation, for with a little practice the laboratory staff can quickly separate the tubes into three lots:—those well below the standard count of 50,000 per ml., those well above the standard, and those which approximate the standard. It is in the latter group only that colonies need to be counted. Experience has shown that some very bad milks can be observed after incubation for 24 hours.

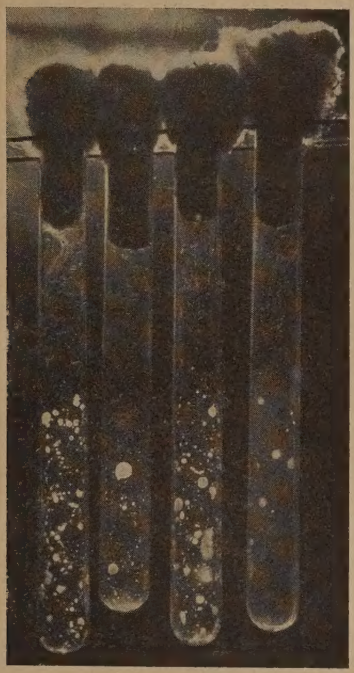

Figure 3.
Tubes Showing Bacterial Development After Incubation.

ADVANTAGES OF THE METHOD.

The method is convenient and requires no great technical skill. Its advantages are twofold, viz.:—

(1) It permits of considerable speed, enabling large numbers of samples to be tested in a short period. A working rate of 80 samples per hour has been found to be a convenient speed for two operators.

(2) There is an appreciable saving in glassware, incubator space and media. Petri dishes, pipettes and diluent are not required, the only glassware necessary being two tubes for each sample. The whirled tubes can be incubated in baskets, thus requiring much less incubator space than plates. About half the quantity of media is required as is necessary for the plate count.

The only additional item of equipment required is the standard loop. It is in connection with this piece of apparatus that the method suffers its greatest disadvantage, viz., its loss of accuracy. This loss, however, can be kept small

if care is taken in the way the loop is used in inoculating. It should be withdrawn vertically from the milk and always at the same speed. In practice the error is accommodated by not penalising milks which approximate the arbitrary standard of 50,000 thermoduric organisms per millilitre.

REFERENCE.

AMERICAN PUBLIC HEALTH ASSOCIATION AND ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. 1948. Standard methods for the examination of dairy products. 9th ed New York: American Public Health Association.

A. H. TUCKER, Government Printer, Brisbane.